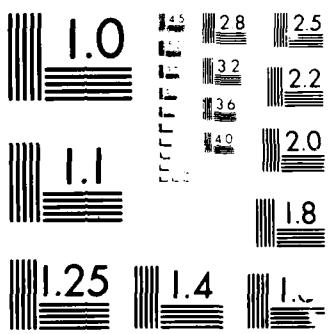


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Genetic and Serological Analysis of *Salmonella* coli Phage  
Hybrids and Development of New Mutator Phages  
with Expanded Host Ranges

Annual/Final Report

September 1, 1987

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<p>Hybrids between <u>Salmonella</u> phage P22 and <u>coli</u> mutator phage Mu have been isolated, using their common hosts <u>E.coli-S.typhimurium</u> recombinants. Although these evolutionary distant phages P22 and Mu have no genetic homology and totally different gene organizations and genomic structures, hybrids were found at an extremely low frequency (<math>10^{-12}</math> or less). The MuimmP22 hybrid class carries the entire late genes of Mu and some early genes at least the <u>c</u> region of P22. P22 grows in strains lysogenic for MuimmP22 though it carries the <u>c</u> region of P22.</p> <p>Mapping analysis of the hybrids by backcrosses with various P22 derivatives revealed that MuimmP22 carries at least the <u>att-C-12</u></p>			
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segment of P22 at the right terminal of the hybrid genome. Mu<sub>immm</sub>P22 type is unable to infect E. coli strains K12 and C while they infect smooth derivatives of E. coli-S. typhimurium WR4028. This observation suggests that the G(+) segment for the Mu tail fiber region is inverted in the Mu<sub>immm</sub>P22 hybrid class and transcribed in inverse G(-) orientation. This hypothesis is supported by a reduced neutralizing activity of an anti-Mu serum. These hybrid phages were, however, unable to induce mutations in the host cells. Therefore, the c and transposase A (and B) genes and/or the inverted termini of Mu have been lost during the process of hybrid formation.

Furthermore, we have isolated and characterized an unusual  $\lambda$ -P22 hybrid carrying the I<sub>m</sub> region of P22 and the c region of  $\lambda$ . We have established an unique method to isolate  $\lambda$ -P22 hybrids carrying the entire late (tail and head coat proteins) genes of  $\lambda$  and some early genes of P22 by plating P22 phage lysates previously grown in a smooth  $\lambda$  lysogen on a rough  $\lambda$  lysogen, WR4027( $\lambda$ ). In such crosses between P22c<sub>2</sub> and  $\lambda$ c+, a new hybrid which forms turbid (c+) plaques on  $\lambda$ c+ lysogens of WR4027 was isolated. E. coli-S. typhimurium strains lysogenic for this new hybrid phage are immune to both  $\lambda$  and P22. Thus this hybrid carries in I<sub>m</sub> region of P22 and the c+ region of  $\lambda$  and is designated as  $\lambda$ I<sub>m</sub>22c<sub>2</sub> $\lambda$  hybrid. Analysis of homology between  $\lambda$ I<sub>m</sub>22c<sub>2</sub> $\lambda$  and P22 revealed that  $\lambda$ I<sub>m</sub>22c<sub>2</sub> $\lambda$  carries the I<sub>m</sub>-9-al-att segment of P22.  $\lambda$ I<sub>m</sub>22c<sub>2</sub> $\lambda$  hybrids can also be isolated from P22- $\lambda$  c+ lysates previously grown on a smooth  $\lambda$  lysogen by plating them on WR4027( $\lambda$ ).



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## SUMMARY

Hybrids between Salmonella phage P22 and coli mutator phage Mu have been isolated, using their common hosts E. coli - S. typhimurium recombinants. Although these evolutionary distant phages P22 and Mu have no genetic homology and totally different gene organizations and genomic structures, hybrids were found at an extremely low frequency ( $10^{-12}$  or less). The MuimmP22 hybrid class carries the entire late genes of Mu and some early genes at least the c region of P22. P22 grows in strains lysogenic for MuimmP22 though it carries the c region of P22.

Mapping analysis of the hybrids by backcrosses with various P22 derivatives revealed that MuimmP22 carries at least the att-c-12 segment of P22 at the right terminal of the hybrid genome. MuimmP22 type is unable to infect E. coli strains K12 and C while they infect smooth derivatives of E. coli - S. typhimurium WR4028. This observation suggests that the G(+) segment for the Mu tail fiber region is inverted in the MuimmP22 hybrid class and transcribed in inverse G(-) orientation. This hypothesis is supported by a reduced neutralizing activity of an anti-Mu serum. These hybrid phages were however unable to induce mutations in the host cells. Therefore, the c and transposase A (and B) genes and/or the inverted termini of Mu have been lost during the process of hybrid formation.

Furthermore, we have isolated and characterized an unusual  $\lambda$ -P22 hybrid carrying the Im region of P22 and the c region of  $\lambda$ . We have established an unique method to isolate  $\lambda$ -P22 hybrids carrying the entire late (tail and head coat proteins) genes of  $\lambda$  and some early genes of P22 by plating P22 phage lysates previously grown in a smooth  $\lambda$  lysogen on a rough  $\lambda$ -lysogen, WR4027( $\lambda$ ). In such crosses between P22c2 and  $\lambda$ c+, a new hybrid which forms turbid (c+) plaques on  $\lambda$ c+ lysogens of WR4027 was isolated. E. coli - S. typhimurium strains lysogenic for this new hybrid phage are immune to both  $\lambda$  and P22. Thus this hybrid carries the Im region of P22 and the c+ region of  $\lambda$  and is designated as  $\lambda$ Im22c $\lambda$  hybrid. Analysis of homology between  $\lambda$ Im22c $\lambda$  and P22 revealed that  $\lambda$ Im22c $\lambda$  carries the Im-9-a1-att segment of P22.  $\lambda$ Im22c $\lambda$  hybrids can also be isolated from P22- $\lambda$ c+ lysates previously grown on a smooth  $\lambda$  lysogen by plating them on WR4027.

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FOREWORD

Though we initially planned for development of a gene cloning vector, we have not established a recombinant DNA method for these hybrid phages during this period.

PROGRESS

A. Genetic Analysis of Hybrids between Salmonella Phage P22 and Coli Mutator Phage Mu.

1. Development of isolation procedures for hybrids between Salmonella phage P22 and coli mutator phage Mu.

A smooth E. coli - S. typhimurium recombinant strain WR4028 is a sensitive host for P22 but resistant to Mu whereas a rough recombinant strain WR4027 is a sensitive host for Mu but resistant to P22. A P22-resistant derivative of WR4028/22 provided hosts for establishing Mu lysogeny. Since these WR4028/22(Mu) and WR4027(Mu) lysogens are rough and resistant to P22, their superinfection with a mixture of rough specific phage(R) led us to isolate smooth revertants designated as NY4028(Mu) and NY4027(Mu) respectively. These smooth Mu lysogens were used for propagation of P22 phage and its genetic interaction with Mu phage. The rough Mu lysogens, WR4028/22(Mu) and WR4027 were used for plating P22 previously grown in the smooth Mu lysogens to select hybrids between P22 and Mu phages.

2. Isolation of hybrids between P22 and Mu.

Although P22 and Mu have no genetic homology and totally different chromosomal gene organizations, we were able to isolate MuimmP22 hybrid class at an extremely low frequency ( $10^{-12}$  or less). To our surprise MuimmP22 hybrid class is able to infect smooth hosts such as WR4028.

These hybrids have not been found in P22 Stocks previously grown in recA strains lysogenic for Mu phages. The recA dependent mechanism and extremely low frequency of hybrid formation suggest that crossovers between P22 and Mu occur through small accidental homologies rather than non-homologous transposition.

3. Immunity response of MuimmP22 hybrid class.

MuimmP22 hybrids can grow in Mu lysogens but not in a rough derivative of P22 lysogen. P22 can grow in strains lysogenic for MuimmP22 though the latter carries the c region of P22.

4. Mapping of MuimmP22 hybrids.

To approximate the length of P22 DNA in MuimmP22 hybrid class which is homologous to P22, backcrosses were performed between these MuimmP22 hybrids and P22 derivatives. Since P22 can

infect WR4028 (MuimmP22) and induce the prophage, backcross P22 recombinants can be scored for mapping the homologous region of these phages.

In superinfection of WR4028 (MuimmP22) with P22c<sub>2</sub>ts<sub>12</sub>, the total frequency of F22 recombinants was approximately 1%. P22 recombinants able to replicate at 40°C, exhibiting the wild type ts<sub>+</sub> phenotype, were scored for the presence of c<sub>+</sub> or c<sub>2</sub> phenotype. Of 887 P22 recombinants with the ts<sub>+</sub> phenotype, 445 also obtained c<sub>+</sub> phenotype (turbid plaques), while 442 retained the c<sub>2</sub> phenotype (clear plaques). The ratio of 445:442 = 1:1 indicates that the left hand homology stretch from the c<sub>2</sub> gene extends a length equal to the distance between c<sub>2</sub> and 12 genes. When we plated the P22 superinfected lysates at 30°C, 243 P22 recombinants expressed the c<sub>+</sub> phenotype were cloned. Each of these clones were tested for their ability to replicate at 40°C, with appropriate 30° controls to determine how many had acquired the gene 12 wild type (ts<sub>+</sub>) phenotype. A total of 20 clones retained ts<sub>12</sub> phenotype while the remaining 223 exhibited the ts<sub>+</sub> phenotype. The ratio of 20: 223 = 1:11 of these recombinants suggests that the right homology stretch from the gene 12 is 11 times of the length between c<sub>2</sub> and 12 genes. However, backcross analysis of MuimmP22 with various F22 amber mutants revealed that P22 genes from the right of the 12 gene are not present in MuimmP22. The high frequency of P22c<sub>+</sub>ts<sub>+</sub> recombinants is therefore due to a consequence of single crossovers suggesting that the right terminal of the hybrid genome ends with P22 homology at the adjacent to the gene 12. Acquisition of the P22 segment at the right arm of the hybrid has resulted in loss of the c-A-B segment at the left of Mu phage. Therefore, the gene organization and genomic structure of the MuimmP22 hybrid class is very similar to that of coliphage P2.

##### 5. Host range and tail fiber antigenicity of MuimmP22 hybrid class.

Although the entire late genes of MuimmP22 hybrid class are derived from those of Mu, MuimmP22 hybrids is unable to form plaques on E. coli strains K12 and C. However, they infect smooth E. coli -S. typhimurium recombinant strains WR4028 and WR4028E. This observation suggests that the G(+) segment for the Mu tail fiber region is inverted in MuimmP22 hybrid class and transcribed in inverse (-) orientation. Changes in amino acid sequence should change not only host ranges but also antigenicity of the tail fiber. MuimmP22 and phages was tested for plaque neutralization with an anti-Mu having k value of about 50 for MuG(+) phage, kindly supplied by Dr. Martha Howe. Slow neutralization (5-fold in 1 hr) of plaque forming ability was observed with a 10-fold diluted serum. This observation supports the hypothesis that the G(+) segment for the tail fiber regions is inverted as G(-) orientation in MuimmP22 hybrid class.

## 6. Lack of mutator activity of MuimmP22 hybrid class.

Mutagenesis by these hybrid phages were tested with maltose, xylose and galactose genes. WR4028 or WR4028E were infected with MuimmP22 and plated on McConkey or FMB agar supplemented with maltose, xylose or galaclose at 30° or 37°. No mutations were detected. This observation suggests that the transposase A and B genes and /or inverted repeat termini of Mu is replaced with the att-c-12 genes of P22 in MimmP22 hybrid class.

## B. Studies of an Unusual $\lambda$ -P22 Phage Hybrid with the $\lambda c$ Region and the Im region of P22

### 1. Isolation of an unusual $\lambda$ -P22 phage Hybrid with the $c^+$ region of $\lambda$ .

Hybrid phages of the  $\lambda$ -P22 class carries the protein coat of  $\lambda$  and contain at least the c region of P22. Such hybrids are isolated from P22 lysates, previously grown on a  $\lambda$  lysogen of a smooth Escherichia coli-Salmonella typhimurium hybrid, WR4028( $\lambda$ ) by plating them on a  $\lambda$  lysogen of a rough strain WR4027( $\lambda$ ). In phage crosses between the clear plaque mutant P22c2 and wild-type  $\lambda c^+$ , a new hybrid  $\lambda$ -P22 type which forms turbid ( $c^+$ ) plaques on  $\lambda c^+$  lysogens of WR4027 was isolated. When this new hybrid type was mixedly infected with  $\lambda c1$ ,  $\lambda c^+$  recombinants were found at a frequency of 4%. Thus although these hybrids carry the  $\lambda c$  region, they are able to grow in  $\lambda$  lysogen.

### 2. Immunity response of hosts lysogenic for $\lambda Im22c\lambda$ .

P22- $\lambda c^+$  hybrids, composed of the P22 coat with at least the c region of  $\lambda$ , are able to form plaques on the smooth host WR4028 ( $\lambda$ ) because they also have second immunity (Im) region of P22. Thus, we suggest that  $\lambda$ -P22 hybrid with the  $\lambda c$  region must also carry the Im region of P22 to be able to form plaques on lysogens. We designated this new hybrid strain as  $\lambda Im22c\lambda$ . Lysogens of WR4028 carrying the new hybrid phage  $\lambda Im22c\lambda$  are immune to both  $\lambda$  and P22.

### 3. Homology between $\lambda Im22c\lambda$ and P22.

When  $\lambda Im22c\lambda$  was backcrossed with a P22 $erf$  mutant by mixed infection of WR4028, P22 $erf^+$  recombinants were recovered at a frequency of about 0.3%. Therefore, this new phage hybrid contains the erf through Im region of P22. Since the attP22 region is located between the erf and Im genes of P22., this hybrid type should be inserted at the prophage intergration site for P22 which has been mapped near proA in S. typhimurium and in

the same location in E. coli K12. We first attempted to lysogenize in E. coli mutant, WR2060, in which a segment including proAproB region as well as the P22~~att~~ site is deleted. We were unable to lysogenize this deletion mutant, suggesting that this hybrid type carries the att region of P22. To confirm this, we tested lysogeny of the above deletion mutant carrying F-lac proA proB<sup>+</sup> attP22 plasmid. As expected, this plasmid carrier was readily lysogenized.

A lysogenic derivative of the plasmid carrier was cultured in nutrient broth overnight at 37°C and then plated on MacConkey lactose agar. Lac- segregants were isolated and tested for lysogeny and the pro<sup>+</sup> phenotype. Of twelve segregants, all were found to be pro- and not lysogenic, indicating that segregants lost the plasmid also lost the prophage. These results establish that this hybrid type integrates at the P22~~att~~ site near the pro region of the bacterial chromosome.

Since both the O-1 antigen conversion gene al and the tail gene 9 of P22 are located between the Im and att regions,  $\lambda$ Im22c $\lambda$  should carry these genes. Although these genes are dispensable for  $\lambda$  phage viability, expression of these genes should be examined. Accordingly the lysogens were tested for somatic O-1 antigen conversion of the host. The expression of Salmonella somatic antigen O-1 in WR4027 lysogens was demonstrated in slide agglutination tests using single factor O-1 antiserum. This result established that this hybrid phage carries and expresses the al gene of P22. To determine whether or not the P22 tail gene 9 is expressed during phage replication, we employed an in vitro assembly method described by Israel, Anderson and Levine (PNAS 57, 284, 1967). Tailless P22~~ts9~~ phage particles were prepared by culturing P22~~ts9~~ at the non-permissive temperature. Such heads contain the entire P22 genome and can be made into infective particles by adding functional tails. When the head preparation was added to a  $\lambda$ Im22c $\lambda$  lysate, incubated for 1hr, and plated on S. typhimurium strain Q at 25°C, a 10,000-fold increased P22 plaque forming activity was observed. Thus the P22 tail gene 9 is expressed though the protein coat and tail of the hybrid phage are those of .

Spontaneous induction of  $\lambda$ Im22c $\lambda$  lysogens occasionally yields phage mutants unable to grow in  $\lambda$  lysogens. Such mutants acquired a bacterial segment substituting for the att-al-9-Im segment of P22 in the  $\lambda$ Im22c $\lambda$  hybrid, and retained, a small segment erf-att region of P22.

#### 4. Isolation of $\lambda$ Im22c $\lambda$ hybrids during crosses between P22- and Phages.

When P22- $\lambda$  lysates previously grown on a smooth  $\lambda$  lysogen were plated on a rough  $\lambda$  lysogen WR4027( $\lambda$ ),  $\lambda$ Im22c $\lambda$  hybrids were readily detected.

Publication:

Yamamoto, N., Droffner, M.L., S., Gemski, P. and Baron, L.S. High frequency by phage hybrids between coliphage 080 and Salmonella phage P22. J. Gen. Virol. 66, 1661-1667 (1985).

Yamamoto, N., Gemski, P. and Baron, L.S. Characterization of Mu<sub>imm</sub>P22 phage, a hybrid between Salmonella phage P22 and coli mutation phage Mu. In preparation, to be submitted to Mol. Gen. Genetics.

Yamamoto, N., Droffner, M.L., Gemski, P. and Baron, L.S. Genomic structure of Im22c phage, an unusual hybrid between Salmonella phage P22 and coli phage lambda, carrying the Im region of P22 and the c region of lambda. In preparation, to be submitted to Mol. Gen. Genetics.

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